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Development and Demonstration of a Superior Method for Microplastics Analysis: Improved Size Detection Limits, Greater Density Limits, and More Informative Reporting

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Abstract

Since the emergence of mass-produced plastics in the 1940s, the global appetite for these materials has been increasing at a rapid rate. Estimates of cumulative plastic waste generated are as much as 6.3 billion metric tons. Only 9% of this material has been estimated to be recycled, while 79% of this material is deposited in landfills and the natural environment. As a result of this, microplastics are now ubiquitous in the environment. Their presence has been detected in surface waters, groundwater sources such as Karst waters, sediments, wildlife, and even consumer products. The major drawback with current efforts in microplastic sample preparation and counting is due to the difference in methods used by researchers. The National Oceanic and Atmospheric Administration (NOAA) was the first to publish a standard method to measure these materials. However, these methods were developed to address large plastic debris in surface water and beach samples. Furthermore, this method can only isolate and account for materials with a density less than 1.2 g/cm^3. Many materials of interest, such as polyvinyl chloride, polyesters, and fluoropolymers, have a density greater than 1.3 g/cm^3 and would therefore be unaccounted for in preparation by this method.

To address the limitations in the current method, a new method has been developed that achieves a lower size detection limit (20 um x 20 um) and greater microplastic density limit (1.8 g/L). In addition, a novel reporting method using detailed size measurements of the microplastic present was implemented. This new approach for data reporting allows one to estimate the mass of microplastics present. Following development, the method was demonstrated with surface waters collected from three locations and fish larvae samples archived by the Illinois Natural History Survey.

Executive Summary

Since the emergence of mass-produced plastics in the 1940s, the global appetite for these materials has been increasing at a rapid rate. Estimates of cumulative plastic waste generated are as much as 6.3 billion metric tons. Only 9% of this material has been estimated to be recycled while 79% of this material is deposited in landfills and the natural environment. As a result of this, microplastics are now ubiquitous in the environment. Their presence has been detected in surface waters, groundwater sources such as Karst waters, sediments, wildlife, and even consumer products.

The major drawback with current efforts in microplastic sample preparation and counting is due to the difference in methods used by researchers. The National Oceanic and Atmospheric Administration (NOAA) was the first to publish a standard method to measure these materials. However, these methods were developed to address large plastic debris in surface water and beach samples. Furthermore, this method can only isolate and account for materials with a density less than 1.2 g/cm^3. Many materials of interest, such as polyvinyl chloride, polyesters, and fluoropolymers, have a density greater than 1.3 g/cm^3 and would therefore be unaccounted for in preparation by this method.

The major steps involved in microplastic sample preparation include initial sieving, digestion of organic matter, density separation, and capture of the materials on a substrate, such as a filter, for counting and identification. The method developed by our group includes these steps; however, modifications have been implemented in the initial sieving and the density separating stages.

To achieve a lower size detection limit, stainless steel sieves with a mesh size of 5 micron were fabricated. These sieves were designed in such a manner so that the mesh screens can be replaced if they are damaged or fouled. Given the reduced pore size of these sieves, vacuum filtration is required to draw the aqueous sample through the unit. Utilizing vacuum filtration could make sieving in the field much more difficult. However, in the laboratory this technique is very common and familiar.

To improve the density separation stage in the standard NOAA method, microplastic isolation units were fabricated. The standard method recommends the use of glass funnels for this step. However, experience with this approach has found that they readily clog, are difficult to rinse free of any remaining polymers stuck to the surfaces and are prone to carry-over of inorganic matter when decanting. The design of the isolation unit was based on specifications reported in the literature for use with sediments. During a research meeting with members of the 100 Plastic Rivers Network, the group explained that this design was flawed due to excess polyvinyl chloride (PVC) contamination from wear of the ball valve utilized. To circumvent this issue, our design of the isolation unit replaces the PVC ball-valve with a stainless-steel gate valve. Initial experiments with this new set-up did discover that after several uses contamination by black particles occurred. However, replacement of the gate valve gaskets with a chemically resistant material (Santoprene) eliminated the problem.

To achieve isolation of denser microplastic types, those greater than 1.2 g/cm^3, parallel experiments were performed with a surface water sample and a laboratory water spiked with microplastics. The three salt solutions investigated included, zinc chloride (1.5 g/L), potassium iodide (1.8 g/L), and cesium formate (2.3 g/L). In addition, sodium chloride (1.2 g/L) was employed as a control. Results from these experiments have shown that potassium iodide (KI) was the best salt solution for density separation. This work has shown that use of zinc chloride and cesium formate as density separation solutions was not amendable to microplastic counting and characterizations. Use of these salt solutions caused precipitation problems and dramatically stained the final filters, and therefore, inhibiting the final microplastic counting and analysis. It was suspected that these salt solutions react with the reagents used in the organic digestion stage before the density separation (most likely the iron catalyst). Potassium iodide did not incur this effect and the filters obtained from final isolation were very clean. Final microplastic counting and analysis could readily be performed in this salt solution as well as samples treated with sodium chloride. The positive controls for the sodium chloride and potassium iodide recovered 89% and 103%, respectively. Although the sodium chloride spike did recover well, it is likely that it is lower due to its lower density than potassium iodide. The polyethylene terephthalate and polyvinyl chloride materials used for spiking, most likely partitioned to the lower fraction during density separation and were discarded with the inorganic material. Given these results, it was determined that potassium iodide was the best salt for density separation because it is amendable to the digestion procedure and achieves a higher density than sodium chloride.

The two surface water samples utilized for the method development portion of this study were obtained from Muskegon Lake, Michigan. They were found to contain 12 and 31 particles per liter form the channel and middle of the lake, respectively. For the middle lake sample, fragments were the most abundant shape detected, followed by fibers. For the channel sample, fibers were the most abundant shape found, followed by fragments. Several bead shaped polymers were also detected in the channel sample, but none were found at the location in the middle of the lake. Green and blue polymers were the most abundant colored polymers detected in the middle lake sample, while clear, blue, and gray colored polymers were the most abundant colored microplastics in the channel sample. For this initial dataset, the longest dimension of each particle was determined. Both the channel and middle lake sits had the most abundant polymers in the 50-200 μm range. This information is important because if the standard NOAA method were employed for these samples, this fraction would be lost in the initial sieving stage of the sample preparation process. Previous work performed in the Muskegon watershed found mean microplastic concentrations near what was observed during this study, in the lake (McNeish, 2015).

In addition to the Michigan surface waters analyzed, a batch of samples were acquired from Missouri surface waters. These samples were collected by Rachel Bartels of the Missouri Confluence Waterkeepers. The particles per liter detected ranged from 8-56. Of the 10 samples analyzed, the Kansas City Riverfront and underneath the Saint Louis Arch samples had the highest concentration of polymers. Like the Michigan samples, these samples also had the longest dimension in the 50-200 μm range. This highlights the importance for lowering the size

detection limit in the initial sieving step. Fragments and fibers were also the most detected polymer types; however, beads were found at much lower abundances in eight out of the ten samples.

After initial reporting of microplastics in these surface water samples, it was realized that there was a fatal flaw in the way microplastics have been previously reported. The conventional unit used to express microplastic concentrations is typically particles per liter or gram. A major issue with this convention of reporting is related to how these materials can change during sample processing. Many of the microplastics collected from the environment are extremely weathered and have a high propensity to break up into more numerous and smaller microplastics. Therefore, this method of reporting is most likely incorrect with regards to what was present in the sample at the time of collection. Furthermore, the unit of particles per liter is of no use with regards to modeling, toxicology, fate, and transport of microplastics in the environment. To provide more valuable information, further reporting of microplastics will include two-dimensional analysis of the materials detected. This method of reporting then gives a means to estimate the mass of each individual microplastics present. Assuming 1 micron in the z-plane and assuming the material is polyethylene (the most abundant plastic), we can use the 2-dimension measurements to calculate the volume of microplastic and therefore estimate its mass. Although this is an approximation, it most likely is a conservative approach and the value calculated is likely a lower limit for mass of plastic present. The information obtained by this approach is extremely valuable. As far has these authors are aware of, no other studies have presented these data in such a way.
Utilizing the developed method and this novel way of reporting, two additional sample sets were processed and reported. The first set of samples processed were archived fish larvae samples obtained from the Illinois State Natural History Survey (INHS). The method developed was first tested on three fish larvae composites. The method developed for surface waters was found to be suitable for this sample type, and therefore, 40 more fish larvae composites were processed and analyzed (~17,000 individual fish larvae). The samples analyzed were not only collected over a large spatial region of the Illinois River, but also over a large temporal region, 2012 to 2019. The occurrence of microplastics in the fish larvae samples ranged from 0.001 particles per individual (0.1%) to 0.11 particles per individual (11%). Using the size data, the estimated mass range of the microplastics found was 0.013 ng (4.5μm x 40μm) to 4,400 ng (1200μm x 3800μm). Further evaluation of these data is underway. A manuscript utilizing these data will soon be drafted and submitted to a peer-reviewed journal.

The last set of samples processed for this project, included 10 surface water collected in Tennessee by the Tennessee Riverkeeper. The detection of polymers in these samples ranged from non-detect to 18 particles per liter. The estimated total mass of polymers in these samples ranged from 1.1 μg to 16 μg. Fragments and fibers were the most abundant shape detected. Beads were detected in two of the samples (1 and 2 counts). Blue, gray, and green polymer colors were the most abundant polymers in these samples. One sample, #1 - Horpeth @ HWY 100, had a very high concentration of black particles. It's interesting to note that this sample appears to be collected near a highway. It is likely that these polymers are the result of tire

wear and/or road run-off. Further investigation of the polymer types would need to be performed to verify this hypothesis.

In conclusion, a method for the analysis of microplastics in surface water samples and fish larvae has been developed at the Illinois Sustainable Technology Center (ISTC). In addition, a novel and valuable method for reporting of area and estimated mass for the polymers detected has also been developed and implemented. Real environmental samples have been processed in this manner and preliminary data for future projects has been generated. The advantages of the ISTC method when compared to the standard NOAA method are a decrease size detection limit (20 μm x 20 μm) and a greater density limit (1.8 g/cm^3).

1. Introduction

Since the emergence of mass-produced plastics in the 1940s, the global appetite for these materials has been increasing at a rapid rate. Estimates of cumulative plastic waste generated are as high as 6.3 billion metric tons Only 9% of this material has been estimated to be recycled while 79% of this material is deposited in landfills and the natural environment. Because of this, microplastics are now ubiquitous in the environment. Their presence has been detected in surface waters, groundwater sources such as Karst waters, sediments, wildlife, and even consumer products (Du, 2020; Panno, 2019; Wang, 2020; Yano, 2020).

The major drawback with efforts in microplastic sample preparation and counting is due to the difference in methods used by researchers. The National Oceanic and Atmospheric Administration (NOAA) was the first to publish a standard method to measure these materials (Masura, 2015). However, these methods were developed to address large plastic debris in surface water and beach samples. Furthermore, this method can only isolate and account for materials with a density less than 1.2 g/cm^3. Many materials of interest, such as polyvinyl chloride, polyesters, and fluoropolymers, have a density greater than 1.3 g/cm^3 and would therefore be unaccounted for when using this method.

The standard NOAA method contains all the elements necessary to prepare samples for microplastic analysis. The sample is first concentrated by initially sieving the material. Then, it is digested to remove organic matter. The digestion technique employed is based on the Fenton reaction, which employs an iron catalyst and hydrogen peroxide. This digestion is necessary to remove any organic matter, which could potentially be counted as a polymer, and any organics bound to the plastics, which in turn could influence the density of the material. This digestion technique has been chosen because it has been shown to remove organic matter yet is not too aggressive to destroy or damage the polymers present.

Following digestion, the sample is then subjected to a density separation stage to remove the inorganic material, such as silica. This stage is also necessary as the denser inorganic material has the potential to be counted as a microplastic. The method uses a salt solution to float the polymers to the top of the solution so that they can be readily decanted and isolated from the inorganic matter at the bottom. Finally, the sample is then passed through a gridded 0.45 μm filter, which allows counting and analyzing the polymers under a microscope. Because this method contains all the required stages to successfully prepare samples for analysis of microplastics, our approach was to develop a method using the standard NOAA method as a template.

2. Methodology

2.1. Fabrication of Apparatus Required

The first stage of the NOAA standard method for analysis of microplastics in aqueous samples involves passing the sample through a series of sieves at sizes ranging from 5.6 mm to 0.3 mm (Masura et al., 2015). The goal of this process is to isolate the material in question and to facilitate downstream processing such as digestion and density separation. Processing large sample volumes directly would be impractical and would consume large quantities of expensive reagents. However, the drawback of this current procedure is that the smallest sieve used is 0.3 mm, which would only allow one to capture microplastics above this size range. Reported size distributions of microplastics in surface waters show that many of the materials present are below this 0.3 mm limit (Koelmans et al., 2019). In addition, this range has been observed in the aqueous and biological samples processed in this study (see Section 3 – Results). To address this size limitation issue, our group has fabricated stainless steel microplastic sieves with a mesh size of 5 μm. Because the materials used are stainless steel, no microplastics contamination can occur from the apparatus. In addition, the sieves have been fabricated to allow changing of the mesh if damage or wear occur. Figure 1 displays one of the sieves in use on the vacuum manifold. Figure 2 presents the technical drawings for the design and materials used. Six of these microplastic sieves have been fabricated for this project. This small mesh size allows for isolated materials that are 60 times smaller than the standard method.

Another portion of the NOAA standard method that required initial modification is the apparatus used for density separation. The NOAA procedure uses a glass funnel with rubber tubing attached to the end. The flow is either restricted or allowed to pass with a clamp. Once the digestate is added to this system, the density solution is then added to the funnel and allowed to settle overnight. After settling, the bottom portion of the solution containing the heavier material can pass through the tubing and is discarded. Finally, the top fraction of the solution is then passed through a 0.3 mm sieve and retained for microplastic counting and characterization.

Preliminary studies performed found this approach problematic due to the difficulty in separating the inorganic heavy fraction from the light/microplastic fraction. Facilitating separation without a magnetic stir bar was unachievable. In addition, it was difficult to rinse microplastic materials stuck to the walls of the funnel and found issues relating to clogging of the small diameter stem of the glass funnel. Through discussions with our international partners at the University of Birmingham, United Kingdom, it became evident of a new design for the density separation systems (Coppock et al., 2017). However, our colleagues also indicated that after several uses and exposure to salt solutions, the separation systems themselves became a source of microplastic contamination. Because this new design had the potential to overcome many of the challenges, a closer look at the new design was taken. During this design review, the PVC ball valve was identified as the most likely source of contamination. Therefore, a similar system was fabricated but instead of a PVC ball valve, a gate valve was incorporated.

The new design did perform well during several test experiments. However, after long-term exposure to the salt solution, black flakes were observed in the blanks as a result to degradation of the O-rings in the gate valve. After replacement of the standard O-rings used in the gate valves with a more chemically resistant material (Santoprene, Manufacturer #1002-7, P/N 27-400-1202), much better performance was achieved, with zero black flakes in the blank. Figure 3 presents the density separation setup used in the NOAA method and density separation unit fabricated at ISTC. The technical drawings for the design and materials used for the density separators are presented in Figure 4. Twelve of these separation units were fabricated for this project. In addition to the modifications to the density separation unit, the only other change made to this portion of the procedure was use of a 0.45 μm gridded filter paper for final capture of microplastics as opposed to a 0.3 mm sieve.

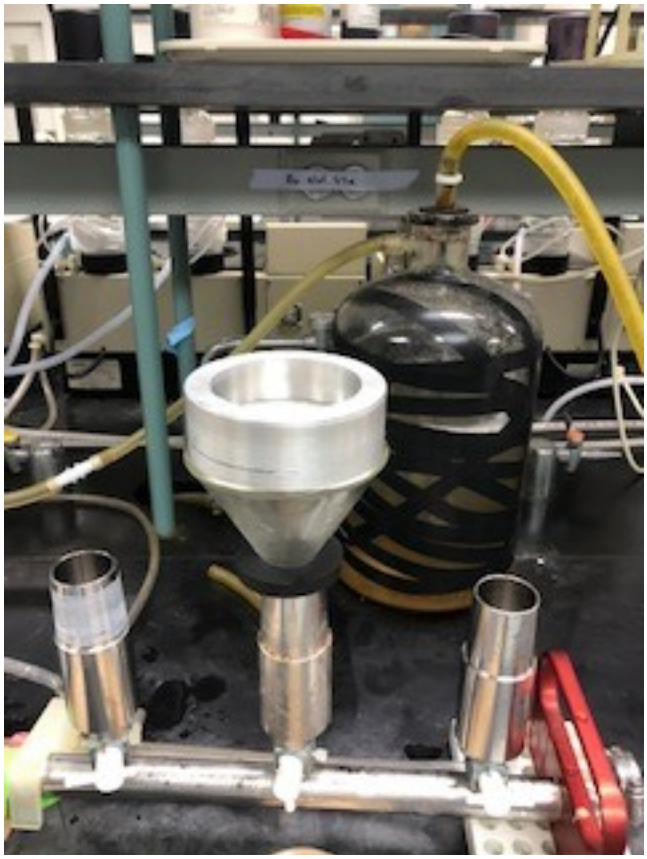


Figure 1. Fabricated Microplastic 5μm Sieve.

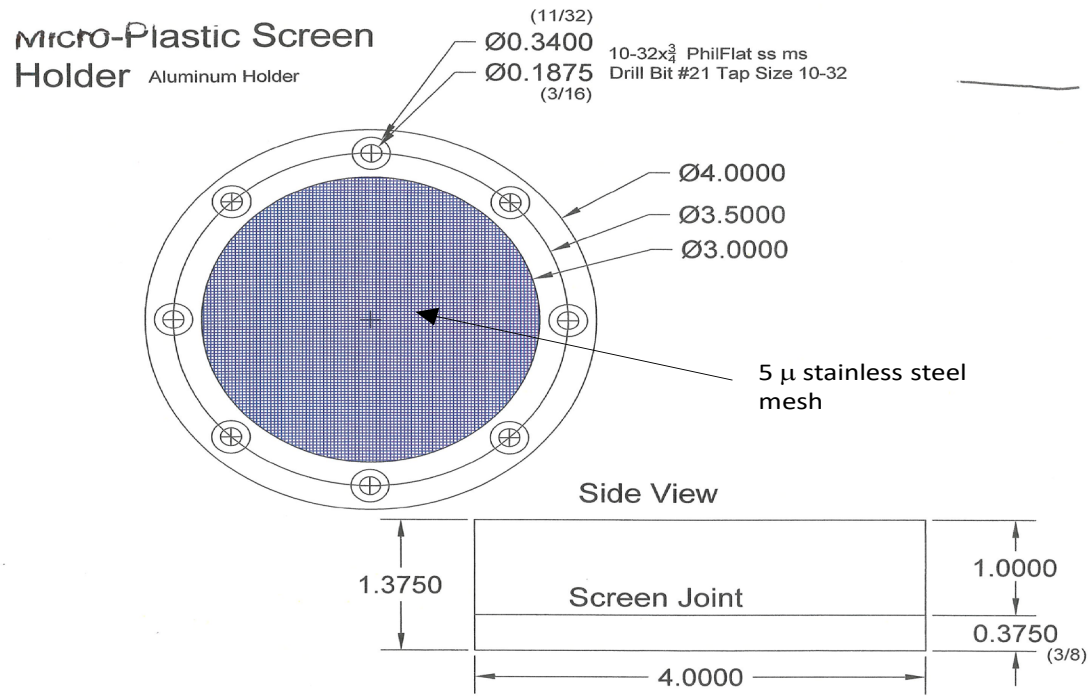


Figure 2. Technical drawing for fabricated microplastic 5µm sieve.

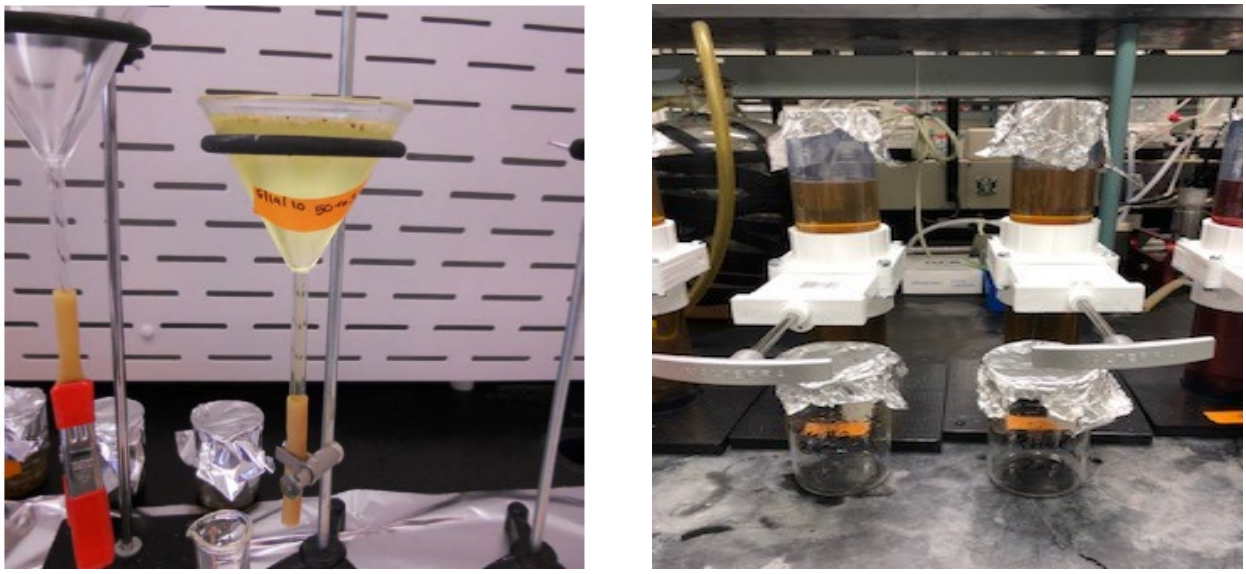


Figure 3. NOAA recommended density separation system (left) and the ISTC fabricated density separation system (right).

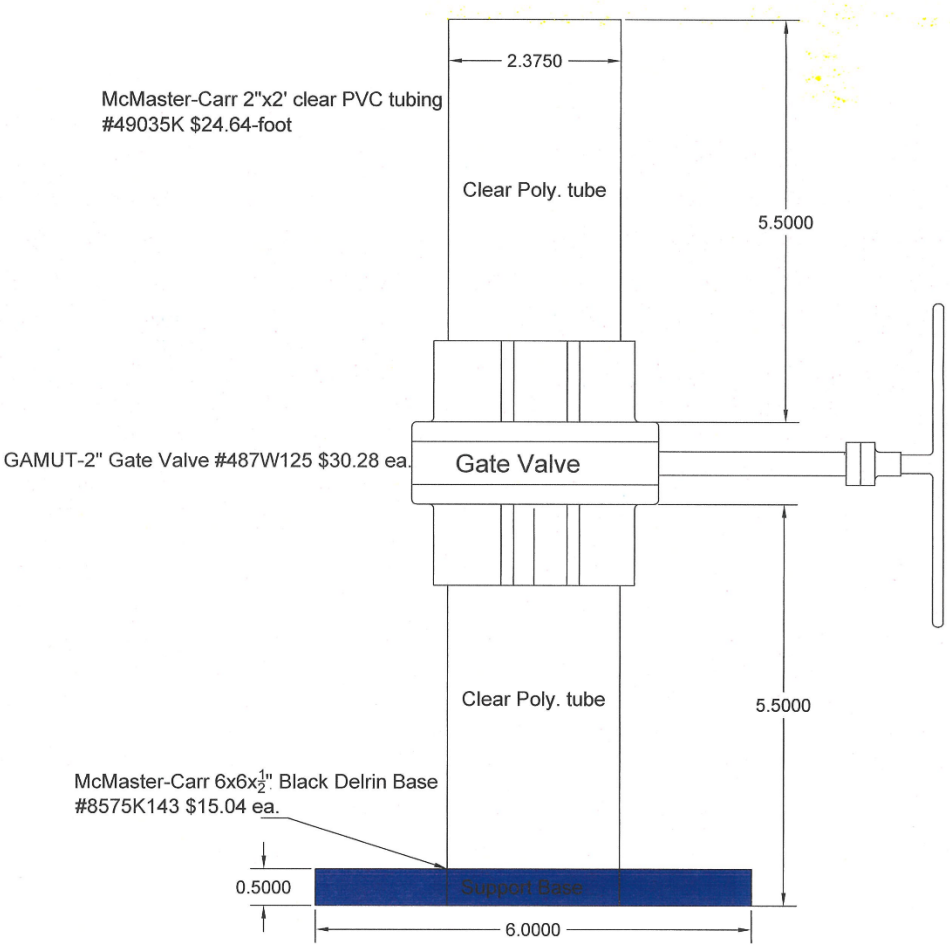


Figure 4. Technical drawing for density separation unit.

2.2. Determination of the Best Suited Salt Solution for Density Separation

To increase the density limit of the NOAA standard method, three different salt solutions were evaluated: zinc chloride (1.5 g/L), potassium iodide (1.8 g/L), and cesium formate (2.3 g/L). In addition, sodium chloride (1.2 g/L) was also used as a control. The water samples used for this portion of the study were collected on May 5, 2017, at Lake Muskegon, Michigan, during sampling for perfluoroalkylated substances during an Illinois-Indiana Sea Grant project. In addition, positive controls containing microplastics spiked into laboratory waters were also processed alongside real samples in each salt solution type. The polymer types used for microplastic spiking included polyethylene (0.91-0.93 g/cm^3), polyamide-Nylon 6 (1.12-1.15 g/cm^3), polyethylene terephthalate (1.33-1.45 g/cm^3), and polyvinyl chloride (1.38-1.55 g/cm^3). These were prepared in-house, had structures like microfibers, and possessed sizes ranging from 250 μm to 500 μm. The samples were processed by the NOAA method with the following modifications:

- Initial sieving to 5 μm with the fabricated stainless-steel sieves.
- Using the fabricated density separation units.
- Collecting the final polymers on a gridded 0.45 μm filter.

Figure 5 displays the final filters obtained from these experiments for procedural blanks and the Lake Muskegon surface water sample. During the density separation, it was observed that the zinc chloride and cesium formate salt solutions produced a brownish-red precipitate. The precipitate was most likely due to reaction with the iron from the digestion stage. This precipitate was captured on filters for both the procedural blank and the zinc chloride samples and rendered counting of microplastics in these samples impossible. The blank filter from the cesium formate sample was clear. However, on the sample filter a precipitate was observed. This precipitate is most likely due to a matrix effect of the environmental samples. Although the potassium iodide filters were slightly stained yellow, both sodium chloride and potassium iodide filters were found to be free of precipitation.

Examination of the filters was performed at the University of Illinois Beckman Institute with a Zeiss SteREO Discovery V20 microscope. From visual observation of the zinc chloride filters, it was obvious that counting of polymers was not possible and therefore these filters were not analyzed. Microscopic evaluation of the cesium filters showed evidence of fouling and accumulation of inorganic matter. It was determined that this salt solution was not amendable to the proposed technique because it was difficult to distinguish between inorganic matter and polymers. Furthermore, there is a concern that microplastics may be co-precipitating out of solution with the inorganic material. The filters from the sodium chloride and potassium iodide solution tests were found to be free of precipitation, and the process of counting, sizing, etc., of the microplastics were amendable to these salt solutions.

The positive controls (microplastic spikes) for the sodium chloride and potassium iodide recovered 89% and 103%, respectively. Although the sodium chloride spike did recover well, the percent recover is lower most likely due to the lower density than potassium iodide. In the sodium chloride solution, the polyethylene terephthalate and polyvinyl chloride most likely partitioned to bottom fraction (higher density) during density separation and was discarded with the inorganic material. Given these results, it was determined that potassium iodide is the best suited salt for density separation because it is amendable to the digestion procedure and achieves a higher density than sodium chloride which would account for a wider range of polymer types detected.

Figure 5. Filters obtained from blanks and surface water samples after density separation.

3. Results

3.1 Demonstration of Method - Surface Water Samples

To demonstrate the newly developed method, surface waters sourced from three locations were processed and analyzed. The locations included Lake Muskegon in Michigan, Missouri, and Tennessee. Given the ubiquitous nature of microplastics, contamination of these materials is quite common and nearly impossible to avoid. The common practice to deal with this issue is to blank subtract all sample results with the counts obtained from procedural blank. All possible efforts to lower background contamination were employed. These efforts included, extensive cleaning and firing of glass sample bottles. In addition, all reagents were filtered multiple times until no microplastics were observed in them. All containers were covered with foil during the sample preparation stages. All glassware and equipment used for sample preparation were cleaned. Four procedural blanks were processed alongside these samples and produced an average microplastics count of 5 particles/liter (Std D = 2 particles/liter). The reported results from each sample preparation batch have been blank subtracted with the appropriate procedural blank. Positive controls used in these experiments consisted of laboratory water spiked with known concentrations and types of microplastics. This quality control parameter is not commonly employed in this research area. However, it is extremely important and further validates the results reported. Implementation of positive controls in type of analysis verifies that no polymer types are lost during the sample preparation process. Three positive controls were processed alongside these samples and produced an average recovery of 115% (Std D = 20%). Table 1 presents the microplastic counts, shapes, and colors detected in the surface waters used in this project.

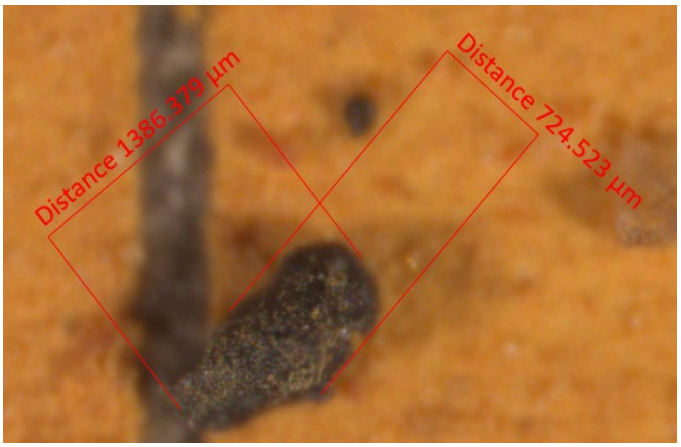
After initial reporting of microplastics in the first two sets of samples, it was realized that there was a fatal flaw in the way microplastics were previously reported. The conventional unit used to express microplastic concentrations is typically particles per liter or gram. A major issue with this convention of reporting is related to how these materials can change during sample processing. Many of the polymers collected from the environment are extremely weathered and have a high propensity to break up into more numerous and smaller polymers. Therefore, this method of reporting is most likely incorrect with regards to what was present in the sample at the time collection. Furthermore, the unit of particles per liter is of no use concerning modeling, toxicology, fate, and transport of microplastics in the environment.

To provide more valuable information, two dimensions of each microplastic detected were measured. Because it is impossible to detect optically how high the particles are off the filter paper, a 1-micron height was used for volume calculations. This method of reporting gives one a means to estimate the mass of each particle present. Assuming the material is polyethylene, the most abundant plastic, these measurements can be used to calculate the volume of the microplastic and therefore estimate its mass. Although this is an approximation, it most likely is a conservative approach and the values calculated are likely a lower limit for mass of plastic present.

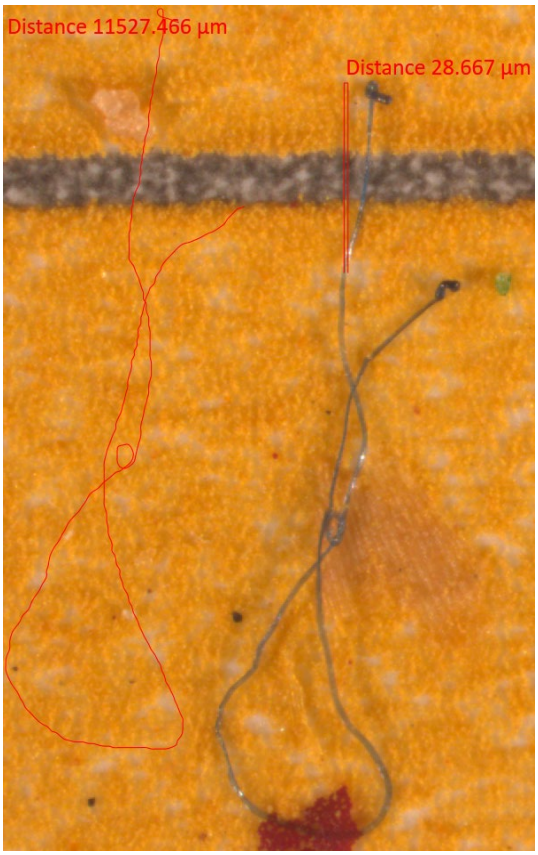
Table 1. Microplastic counts, shapes, and colors detected in surface water samples (in units of particles detected).

SAMPLE_ID	SAMPLE_ LOCATION	SAMPLE_TYPE	Fiber	Fragment	Pellet (Bead)	Foam	Film	Conc. (No/L)	Blue	Clear	Clear Bead	White	Red	Gray	Black	Purple	Beige	Pink	Yellow	Orange	Green
Channel	Channel between Lake Michigan and Muskegon Lake	Michigan Surface Water	11	7	1	0	0	**12**	3	8	1	1	0	3	0	0	1	0	0	1	0
Lake	Middle of Muskegon Lake	Michigan Surface Water	18	20	0	0	0	**31**	12	1	0	1	0	2	4	0	0	2	1	0	14
#3	Gasconade	Missouri Surface Water	6	10	1	0	0	**14**	0	2	0	2	2	0	2	1	0	3	2	3	0
#10	Portage	Missouri Surface Water	6	5	0	0	0	**8**	2	2	0	0	0	0	0	2	1	1	1	0	1
#7	KC Castlewood	Missouri Surface Water	8	12	1	0	0	**18**	3	5	1	1	0	0	7	1	1	0	1	1	0
#6	GEO Winter	Missouri Surface Water	9	3	1	0	0	**10**	4	1	0	1	0	0	1	0	0	4	0	0	0
#4	Big	Missouri Surface Water	12	6	0	0	0	**15**	4	6	0	1	0	0	1	0	3	1	1	1	0
#1	I-KAW	Missouri Surface Water	5	16	5	0	0	**23**	1	1	0	9	1	3	2	1	2	3	1	0	0
#5	Pour B	Missouri Surface Water	18	17	2	0	0	**34**	3	2	0	5	6	1	1	3	7	1	0	4	4
#2	KC Riverfront	Missouri Surface Water	19	38	2	0	0	**56**	2	4	0	18	6	1	11	0	6	0	0	7	3
#8	ARCH	Missouri Surface Water	16	34	2	0	0	**49**	8	3	0	10	5	4	3	4	6	2	0	1	3
#9	St. Charles	Missouri Surface Water	9	15	2	0	0	**23**	2	2	0	3	2	1	2	1	5	1	1	0	3
TN #1	Horpeth @ HWY 100	Tennessee Surface Water	9	7	2	0	0	**13**	3	1	0	0	0	3	10	0	0	1	0	0	0
TN #2	Richland Cr@ Clarendor Ave	Tennessee Surface Water	1	3	0	0	0	**non-detect**	0	2	0	0	0	2	0	0	0	0	0	0	0
TN #3	Cumberland @ Cleeces FWY S.	Tennessee Surface Water	7	2	0	0	0	**4**	4	0	0	0	0	2	2	1	0	0	0	0	0
TN #4	Cumberland @ Shelby Pk Ramp	Tennessee Surface Water	1	3	0	0	0	**non-detect**	0	0	0	0	0	1	1	0	0	1	0	0	1
TN #5	Stones River@ HWY 70	Tennessee Surface Water	4	9	0	0	0	**8**	3	1	0	0	0	2	1	1	0	1	0	0	4
TN #6	Browns Cr @ Visco Dr	Tennessee Surface Water	2	4	1	0	0	**2**	2	0	0	0	0	1	2	0	0	0	0	0	2
TN #7	Whites Cr@ Hospital Rd	Tennessee Surface Water	2	2	0	0	0	**non-detect**	0	0	0	0	0	1	0	0	0	0	0	0	3
TN #8	Long Branch @ Sukin House	Tennessee Surface Water	5	6	0	0	0	**6**	3	1	0	0	0	4	0	1	0	0	0	0	2
TN #9	Cumberland @ Clarksville	Tennessee Surface Water	3	11	0	0	0	**9**	1	0	0	0	0	5	0	2	0	0	0	0	6
TN #10	Decatur, AL @ Riverwalk Marina	Tennessee Surface Water	6	5	0	0	0	**6**	3	2	0	0	0	1	1	0	0	1	0	0	3

Figure 6 presents two images of microplastics detected in Tennessee surface waters with their measured dimensions. Table 2 presents the total estimated mass of microplastics detected in 1 liter of surface water for the sites collected in Tennessee. Also contained in this table are the average estimated microplastic masses and ranges.



Sample TN#1
Fragment
Estimated Mass: 0.93 µg



Sample TN#9
Fiber
Estimated Mass: 0.31 µg

Figure 6. Select Images of microplastics detected in Tennessee surface waters with two-dimensional measurements.

Table 2. Estimated mass information for Tennessee surface water samples. Total mass estimate is per liter.

SAMPLE_ID	SAMPLE_ LOCATION	SAMPLE_TYPE	Total Mass Estimate, µg	Average Mass Estimate, µg	Mass Estimate Range, µg
TN #1	Horpeth @ HWY 100	Tennessee Surface Water	16	0.90	0.018 - 11
TN #2	Richland Cr@ Clarendor Ave	Tennessee Surface Water	2.1	0.52	0.153 - 0.917
TN #3	Cumberland @ Cleeces FWY S.	Tennessee Surface Water	3.0	0.33	0.068 - 1.4
TN #4	Cumberland @ Shelby Pk Ramp	Tennessee Surface Water	1.9	0.49	0.11 - 1.5
TN #5	Stones River@ HWY 70	Tennessee Surface Water	8.0	0.67	0.065 - 3.2
TN #6	Browns Cr @ Visco Dr	Tennessee Surface Water	1.2	0.17	0.093 - 0.45
TN #7	Whites Cr@ Hospital Rd	Tennessee Surface Water	1.1	0.28	0.087 - 0.47
TN #8	Long Branch @ Sukin House	Tennessee Surface Water	2.7	0.24	0.055 - 0.57
TN #9	Cumberland @ Clarksville	Tennessee Surface Water	3.6	0.25	0.058 - 0.44
TN #10	Decatur, AL @ Riverwalk Marina	Tennessee Surface Water	2.7	0.25	0.048 - 0.56

3.2 Demonstration of Method - Fish Larvae Samples

Given the success of the microplastics method developed for aqueous samples, the next media tested was biological tissues (fish larvae). Fish larvae are a great monitoring species for microplastic studies. They may be more sensitive to environmental contaminants than later life-stages of fish, are present at the lower tropic levels, and tend to have high prey consumption rates during their development stages. Fish larvae are also an ideal test subject for microplastics exposure because large numbers (500-1000) can be analyzed in a single composite sample. Even more exciting is that the Illinois Natural History Survey (INHS) has a vast archive of these samples collected all along the Illinois river dating back to the last ten years. Therefore, processing and analyzing these samples provided a large spatial and temporal picture (2012-2019) of the exposure of microplastics in fish larvae, while at the same time allowing us to reduce drastically the cost for sample collection. Figure 7 displays an image for two species of fish larvae used in this study.

The method used for these samples was identical to the one used for aqueous samples. A test experiment with two fish larvae composites (600 and 1000 individuals) showed that the method was amendable with this type of biological tissue. The only noteworthy difference was that longer digestion times were required due to the large amount of organic mass present. Thirty-eight more fish larvae composites were processed and analyzed (~17,000 total individual fish larvae).

The occurrence of microplastics in the fish larvae samples ranged from 0.001 particles per individual (0.1%) to 0.11 particles per individual (11%). Using the size data, the estimated mass range of the polymers found was 0.013 ng (4.5µ x 40µ) to 4,400 ng (1200µ x 3800µ). Figure 8 presents two images of the microplastics detected in the fish larvae samples and Table 3 contains the microplastic counts per individual and the mass estimate information.

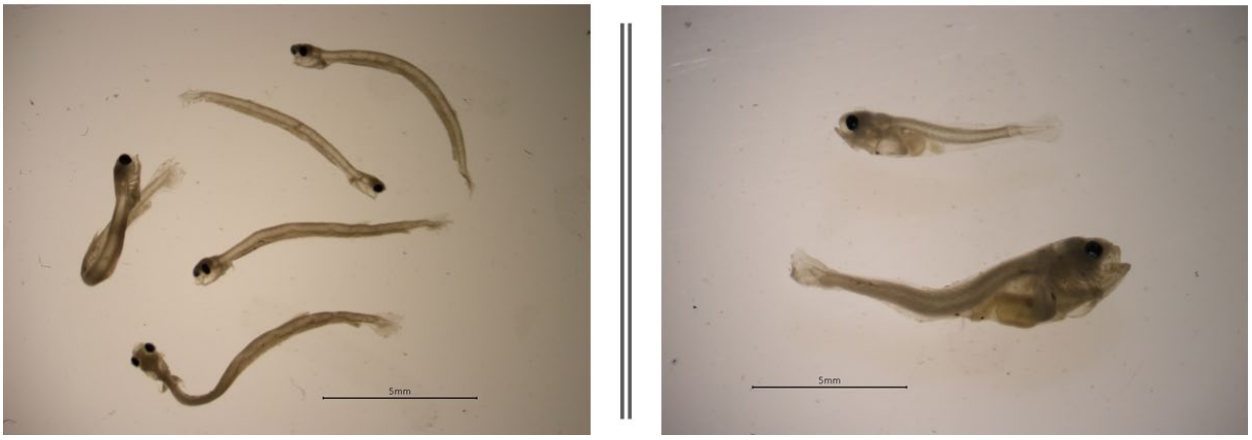


Figure 7. Images of select fish larvae processed and analyzed in this study.

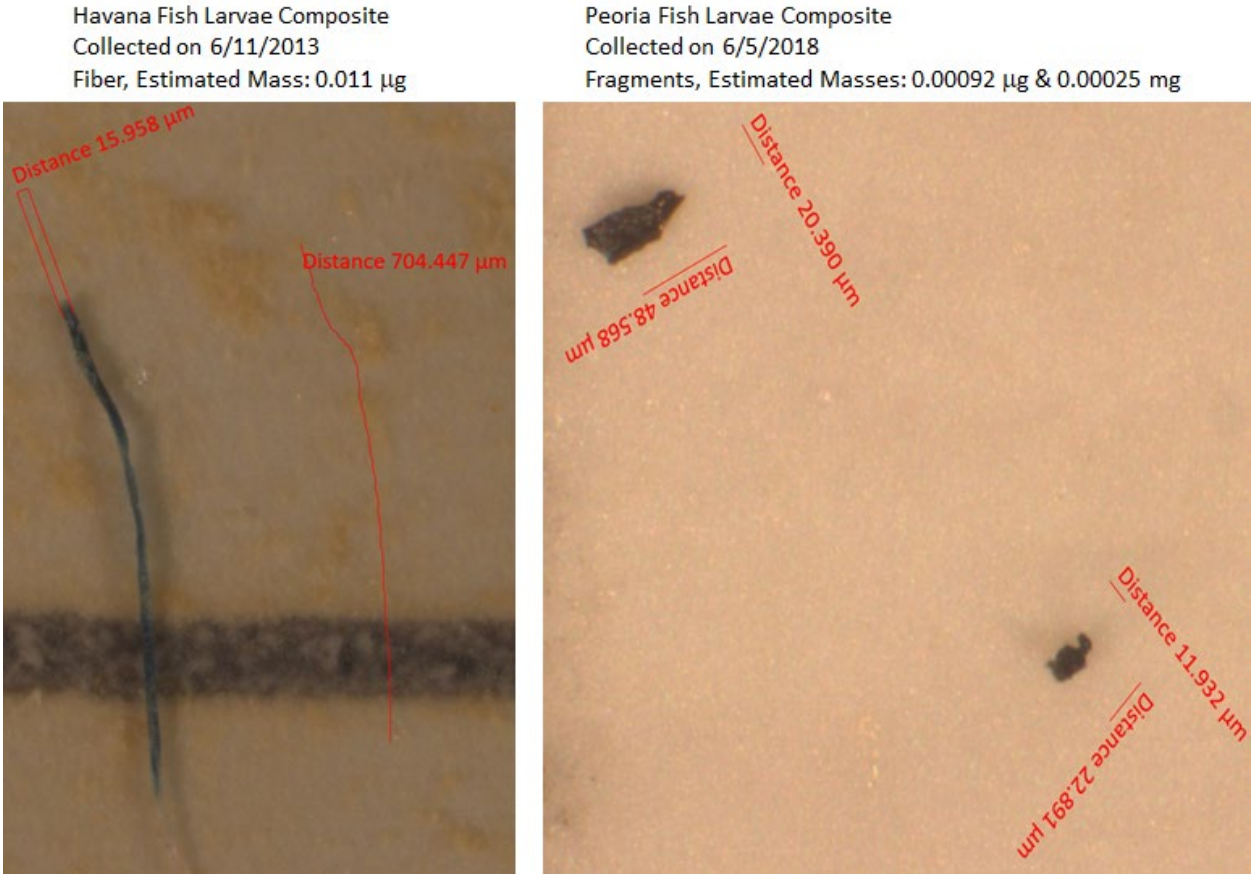


Figure 8. Select images of microplastics detected in fish larvae samples with two-dimensional measurements.

Table 3. Microplastic counts per fish larvae individual and mass estimate information.

Sample ID	n=	Sample Location	Date	Conc. (#particles /# individual)	Average Mass Estimate, µg	Mass Estimate Range, µg
Fish Larvae Batch Chute, 06/ 27/2013	1000	Batch Chute	6/27/2013	0.006	0.013	0.0018 - 0.060
Havana, 06/11/2013	837	Havana	6/11/2013	0.007	0.013	0.0010 - 0.046
Havana, 06/15/2017, 1 of 3	211	Havana	6/15/2017	0.062	0.43	0.073 - 1.8
Havana, 06/15/2017, 2 of 3	195	Havana	6/15/2017	0.103	0.36	0.017 - 0.84
Havana, 06/15/2017, 3 of 3	181	Havana	6/15/2017	Non-Detect	0.00034	0.000089 - 0.00036
Havana, 06/15/2017, 1 of 9	146	Havana	6/15/2017	0.048	0.29	0.021 - 0.95
Havana, 06/15/2017, DR, 2 of 9	106	Havana	6/15/2017	Non-Detect	5.40E-05	0.000013 - 0.00014
Havana, 06/15/2017, DR, 3 of 9	89	Havana	6/15/2017	0.045	0.0079	0.00042 - 0.028
Havana, 06/15/2017, DR, 4 of 9	131	Havana	6/15/2017	0.107	0.017	0.00059 - 0.086
Havana, 06/15/2017, DR, 5 of 9	145	Havana	6/15/2017	0.014	1.90E-02	0.00056 - 0.063
Havana, 06/15/2017, DR, 6 of 9	165	Havana	6/15/2017	Non-Detect	0.51	0.11 - 0.94
Havana, 06/15/2017, DR, 7 of 9	142	Havana	6/15/2017	0.056	1.2	0.11 - 4.4
Havana, 06/15/2017, DR, 8 of 9	156	Havana	6/15/2017	0.038	0.61	0.097 - 1.7
Havana, 06/15/2017, DR, 9 of 9	31	Havana	6/15/2017	Non-Detect	0.3	0.17 - 0.37
Havana, 06/05/2018	698	Havana	6/5/2018	0.023	0.0069	0.000037 - 0.032
Havana, 06/10/2019, DL	990	Havana	6/10/2019	0.001	0.025	0.0034 - 0.085
Henry, 05/24/2017	537	Henry	5/24/2017	0.02	0.042	0.00064 - 0.42
Henry, 06/10/2019 UL	994	Henry	6/10/2019	0.015	0.0079	0.00016 - 0.057
Lily Lake, 05/30/2012, BWUR, 1 of 4	253	Lily Lake	5/30/2012	0.091	0.015	0.000081 - 0.11
Lily Lake, 05/30/2012, 3 of 4	259	Lily Lake	5/30/2012	0.004	0.0094	0.00016 - 0.065
Lily Lake, 06/30/2012, 4 of 4	238	Lily Lake	6/30/2012	0.004	0.011	0.00086 - 0.025
Lily Lake, 06/10/2013	1009	Lily Lake	6/10/2013	0.009	0.038	0.0010 - 0.37
Lily Lake, 06/24/2014	332	Lily Lake	6/24/2014	0.015	0.0079	0.00064 - 0.052
Lily Lake, 06/15/2017	180	Lily Lake	6/15/2017	0.028	0.015	0.00082 - 0.082
Lily Lake, 06/28/2017	443	Lily Lake	6/28/2017	0.014	0.15	0.027 - 0.43
Lily Lake, 06/06/2018, BWL	365	Lily Lake	6/6/2018	0.09	0.012	0.00018 - 0.083
Lily Lake, 06/06/2018	1126	Lily Lake	6/6/2018	0.02	0.014	0.00059 - 0.056
Lily Lake, 06/06/2018, BWR	365	Lily Lake	6/6/2018	0.014	0.017	0.00036 - 0.15
Matanzas, 06/11/2013	753	Matanzas	6/11/2013	Non-Detect	0.033	0.0011 - 0.091
Matanzas, 06/15/2017	345	Matanzas	6/15/2017	0.017	0.024	0.000056 - 0.22
Matanzas, 06/05/2018, DR, 1 of 4 (Split 1)	303	Matanzas	6/5/2018	0.053	0.017	0.00079 - 0.052
Matanzas, 06/05/2018, DR, 1 of 4 (Split 2)	216	Matanzas	6/5/2018	0.005	0.018	0.00024 - 0.15
Matanzas, 06/05/2018, DR, 3 of 4	221	Matanzas	6/5/2018	0.036	0.017	0.00084 - 0.084
Matanzas, 06/05/2018, DR, 4 of 4	255	Matanzas	6/5/2018	0.035	0.015	0.00031 - 0.059
Peoria L&D, 06/11/2013	1031	Peoria	6/11/2013	0.002	0.0086	0.00080 - 0.022
Fish Larvae Peoria Lock and Dam, 05/01/2015	600	Peoria	5/1/2015	0.008	0.015	0.0045 - 0.034
Peoria, UL, 06/01/2016	315	Peoria	6/1/2016	0.057	0.032	0.00063 - 0.14
Peoria DL, 06/01/2016	140	Peoria	6/1/2016	0.071	0.011	0.00022 - 0.049
Peoria UL, 06/1/2016	283	Peoria	6/1/2016	0.046	0.011	0.000087 - 0.090
Peoria L&D, 05/25/2017	506	Peoria	5/25/2017	Non-Detect	0.064	0.0028 - 0.22
Peoria, 06/03/2014	386	Peoria	6/3/2018	0.021	0.0093	0.00010 - 0.041
Peoria, 06/05/2018	231	Peoria	6/5/2018	0.009	0.23	0.062 - 0.55

4. Discussion

Microplastic counts in the surface waters analyzed in this study ranged from non-detect to 56 particles per liter. On a cautionary note, microplastics occurrence is highly dependent on many factors and can vary greatly not only on a spatial scale, such as what is observed in the Lake Muskegon surface water samples, but also on a temporal scale, such as what is observed in the fish larvae samples. Many factors such as land use, precipitation events, stream flows, vertical distribution in the water column, and water temperature can influence how and where microplastics occur. The results obtained in this study, and most others, are only a single snapshot for the presence of microplastics. To provide a better understanding of how these materials occur in the natural environment, long-term monitoring of select sites is required.

Figures 9 and 10 present the largest dimension measured for microplastics detected in the Lake Muskegon and Missouri surface waters, respectively. These data only consider the longest dimension of these materials and, therefore, are not a true representation of the size of these materials. In practice, researchers should strive to obtain at least one or two additional size measurements of the microplastics detected to provide a more accurate description of these materials. The significance of these figures is that they demonstrate that the most abundant microplastics detected in these surface waters, those less than 300 μm, would be missed if they were processed by the standard NOAA method. This highlights the importance that researchers should strive for the lowest size detection when measuring microplastics in environmental samples. Given the modifications made to the standard NOAA method in this study, a better understanding of microplastics in surface waters has been achieved.

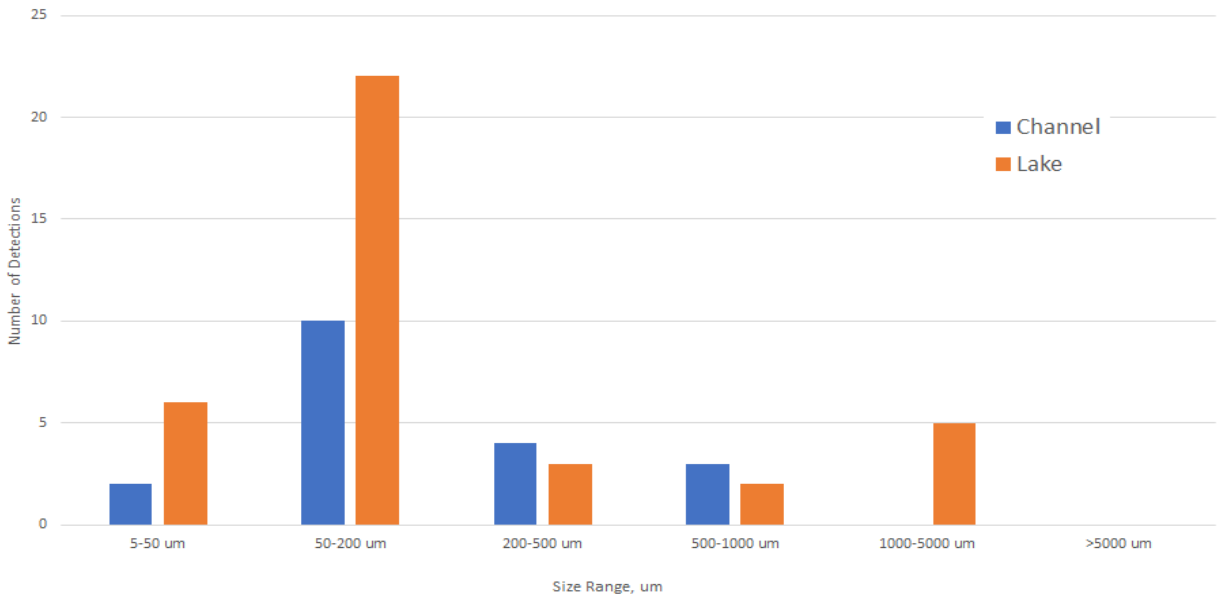


Figure 9. Size distribution of longest dimension measured for microplastics detected in Muskegon Lake surface.

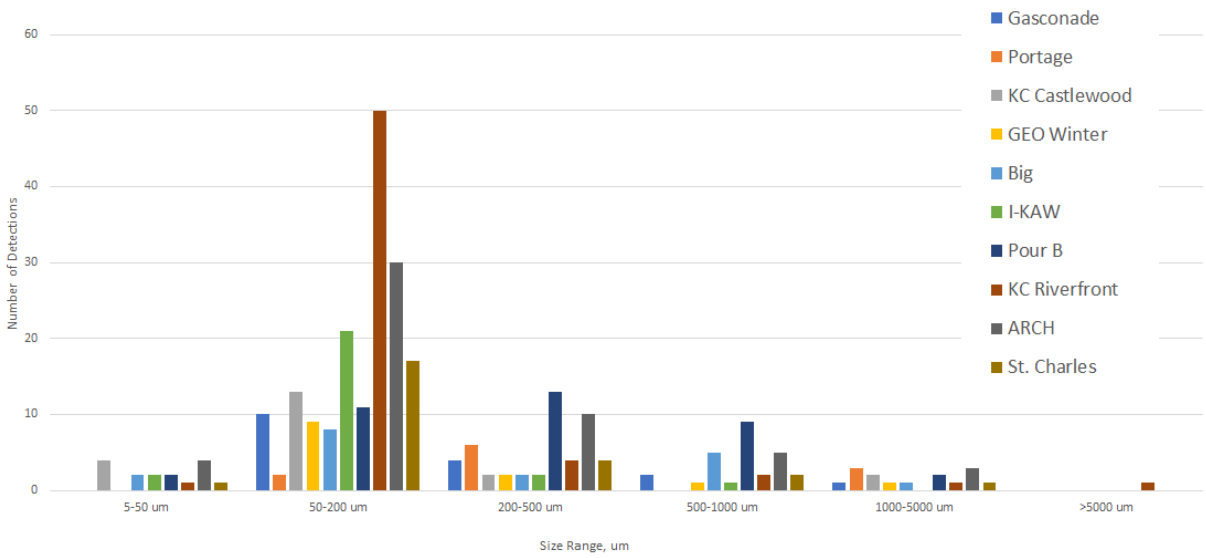


Figure 10. Size distribution of longest dimension measured for microplastics detected in Missouri surface water samples.

Reporting the shapes of microplastics detected can be a useful piece of information with regards to the source, transport, fate, and potential impacts to wildlife exposed to them. For example, the presence of microbeads could be an indicator for personal care products as a source of these materials. Additionally, the abundance of fibers could suggest textile washing as a potential source, and an abundance of fragments could suggest the breakdown of larger plastic materials as the source. In addition to information regarding source, the shape of microplastics could also be an indicator of how these materials could potentially impact wildlife. For example, a microbead could readily be excreted by an organism that ingests it. However, a fiber or fragment could become entrained in the digestion system because of their irregular shape as compared to the relative uniformity of a microbead (De Sales-Ribeiro, 2020). Figures 11-14 present the shape distribution of the microplastics detected in surface waters and in fish larvae. Fibers and fragments were by far the most abundant materials detected. Microbeads were commonly detected; however, their abundances were very low when compared to fibers and fragments.

Color is also a useful piece of information when reporting microplastics. It could provide clues to the source of these materials and wildlife may preferentially consume specific materials of certain colors. In addition, this property of the polymers can be readily noted during analysis of the samples. Figures 15-18 present the color distribution of the microplastics detected in surface waters and fish larvae. Of all the surface waters, blue was the most common color (15%), followed by white/black/green (12%), and then clear (11%). Yellow was the least common color detected (2%). For the fish larvae, blue was also the most common color detected (22%), followed by black (20%) and clear (19%). Beige and yellow were the least common colors detected in the fish larvae (1%).

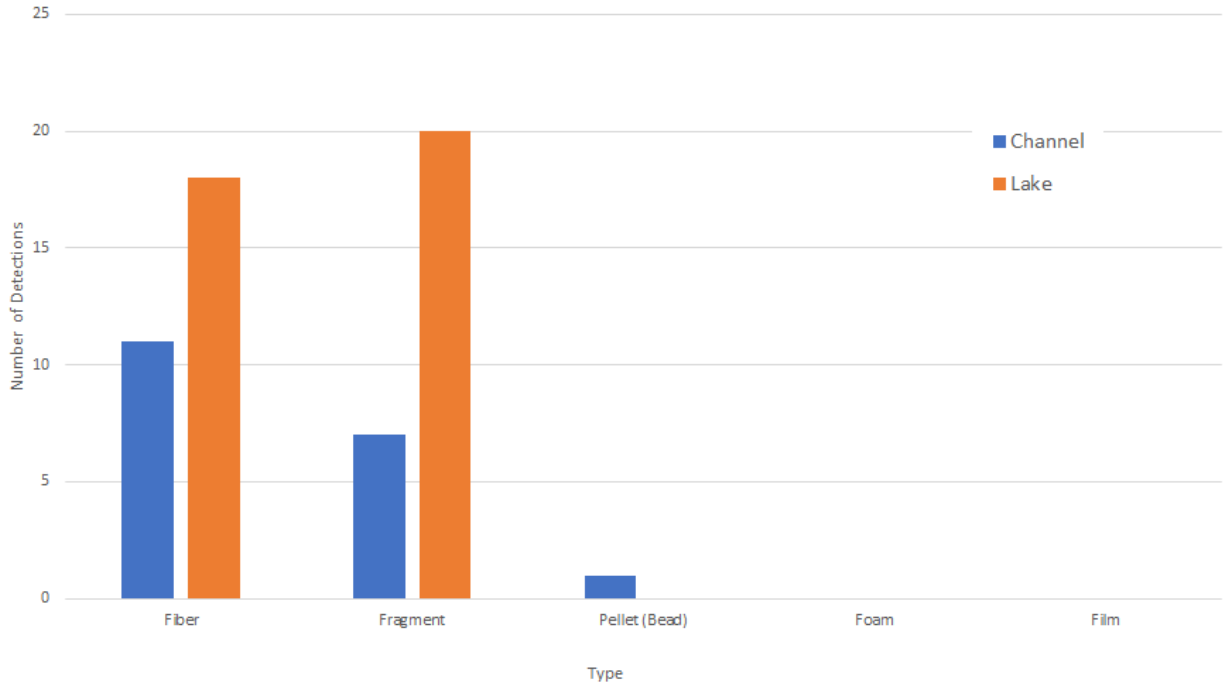


Figure 11. Shape distribution of microplastics detected in Muskegon Lake surface water samples.

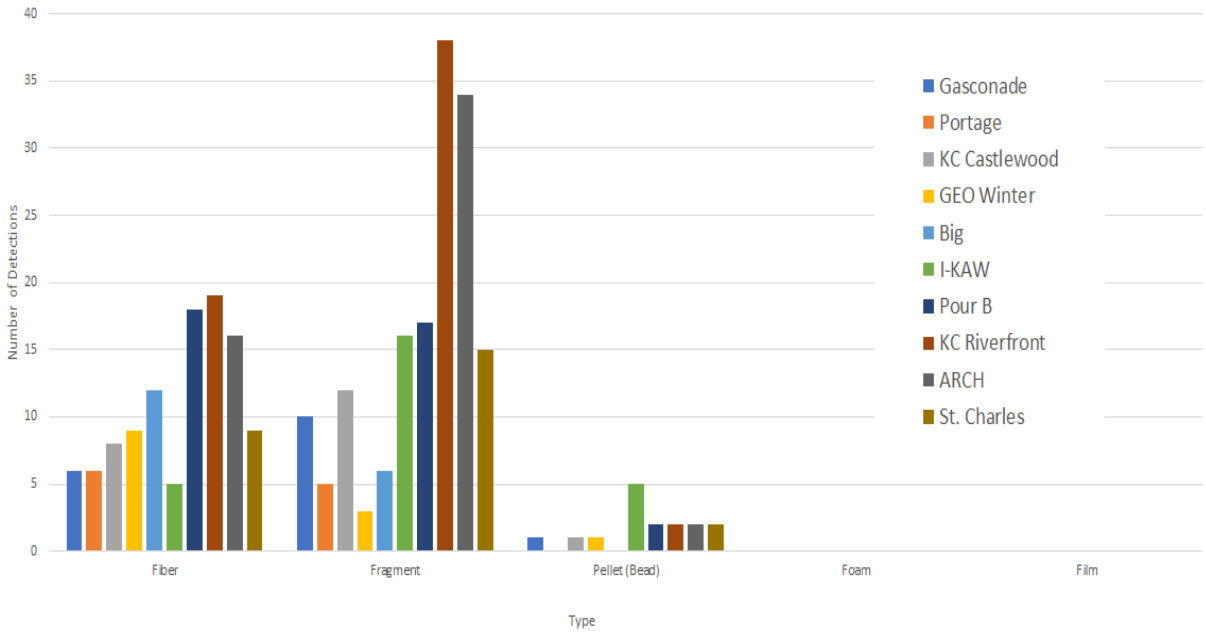


Figure 12. Shape distribution of microplastics detected in Missouri surface water samples.

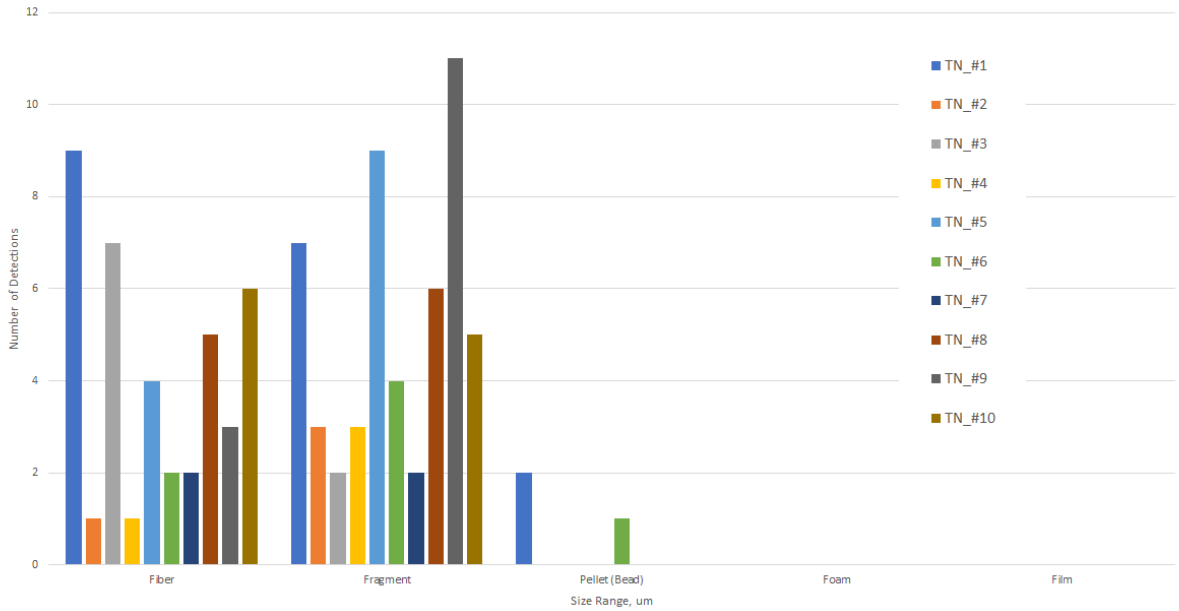


Figure 13. Shape distribution of microplastics detected in Tennessee surface water samples.

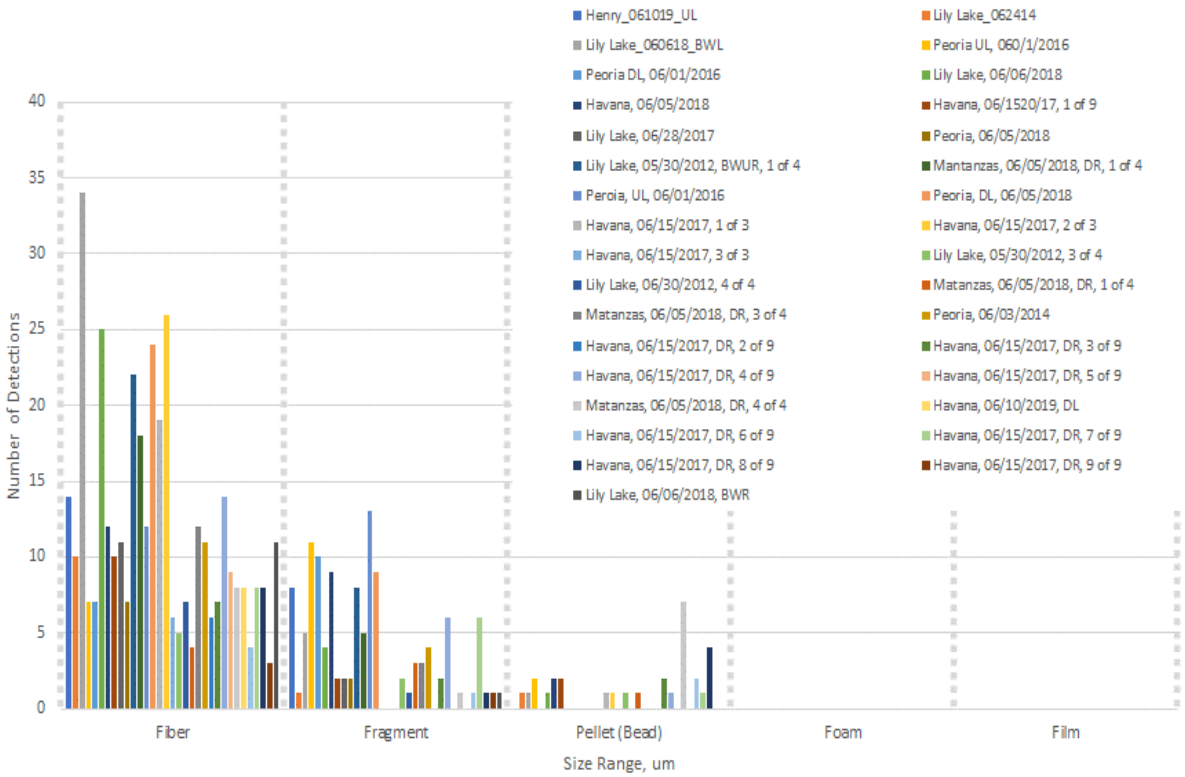


Figure 14. Shape distribution of microplastics detected in fish larvae samples.

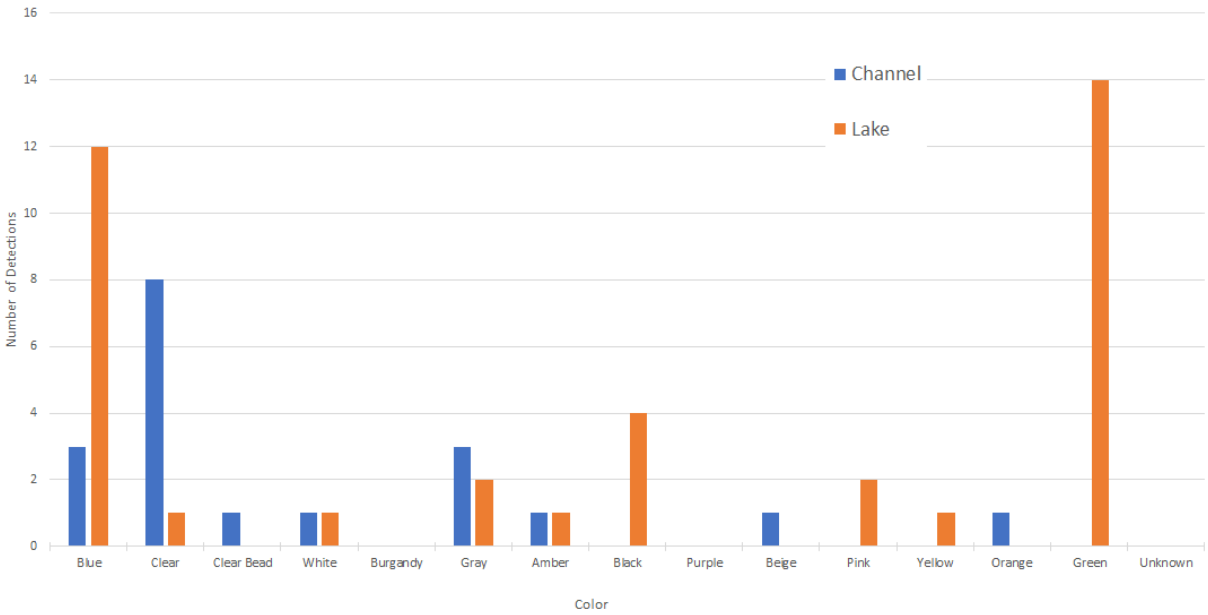


Figure 15. Color distribution of microplastics detected in Muskegon Lake surface water samples.

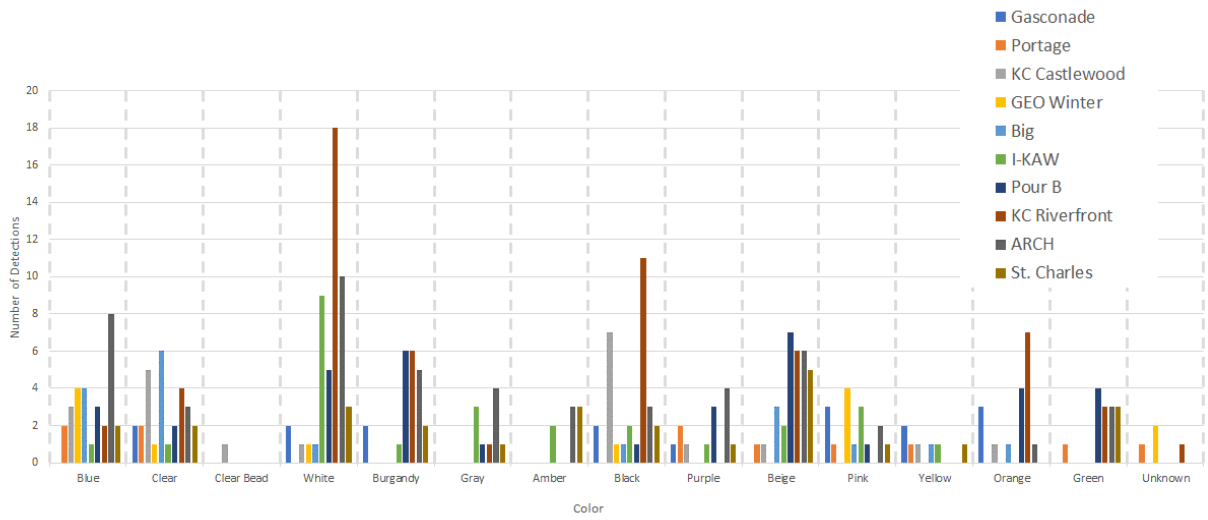


Figure 16. Color distribution of microplastics detected in Missouri surface waters.

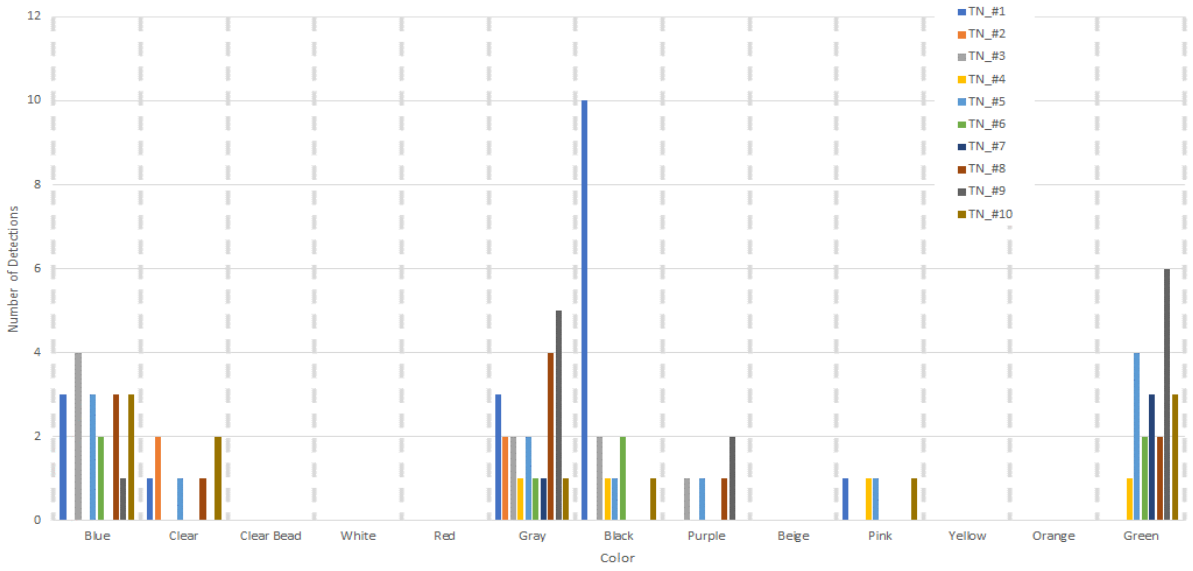


Figure 17. Color distribution of microplastics detected in Tennessee surface waters.

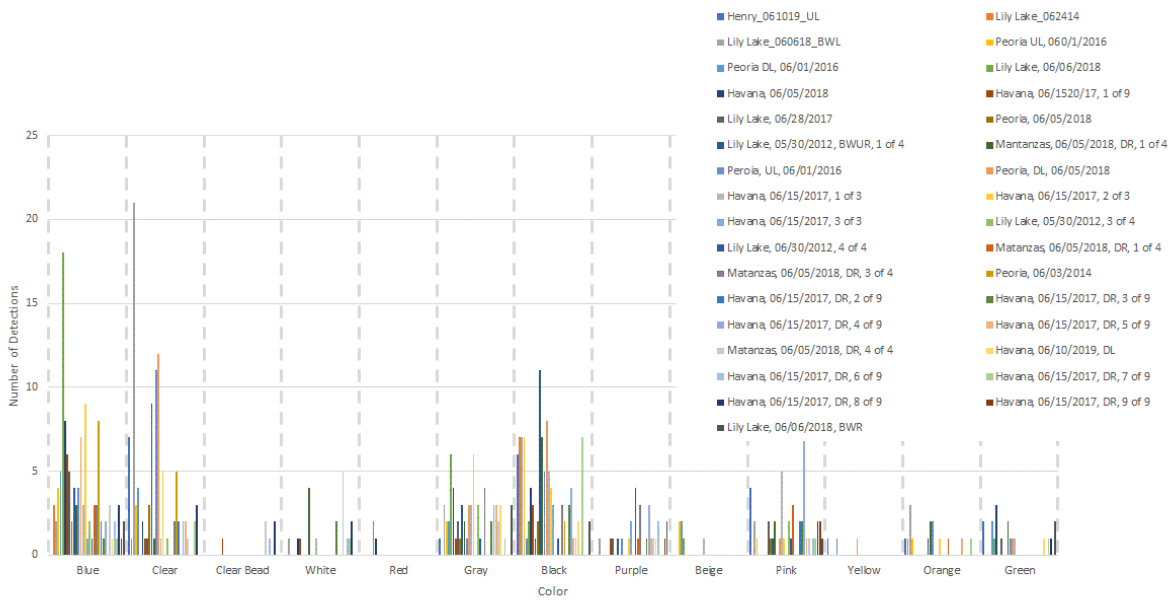


Figure 18. Color distribution of microplastics detected in fish larvae.

With regards to the mass estimates calculated in this study, there is no means for comparison to what has been reported in the literature because this style of reporting has not been performed previously. Further reporting for microplastics will continue to include the size measurements and estimated masses. This information will be extremely valuable to the research community and will give a unique and competitive edge over others in this area. Mass estimation of the microplastics detected could certainly overcome the current flaws in particle per unit reporting. Even if a polymer breaks up into smaller particles during the sample preparation process, the total mass will not change. Furthermore, obtaining estimates to the mass of plastic present provides the information needed to fully address this emerging contaminant. A prime example of this was the work done with the fish larvae. With a handle on the mass of plastic associated with these organisms, it is understood how these organisms can be affected by their exposure.

Many studies exist that have looked at microplastics in large sport fish or commercial fish. However, few studies have addressed fish larvae. Given the small size of fish larvae, the ratio of microplastic mass to fish larvae mass would be expected to be much greater than those found for larger, later-in-life fish. Therefore, the contaminant burden of microplastics on fish larvae would be expected to be much greater. Ideally, development of a method to directly measure the mass of these materials is required for a more accurate assessment. However, many technical challenges will need to be overcome. Several of these major challenges will include measuring extremely small masses (the microgram to picogram range) and total elimination of non-polymer material (such as residue organic matter). Finding a technique to measure accurately the mass of microplastics in environmental samples will continue to be a goal for which our group will strive.

5. Conclusion

Development of method to analyze microplastics in environmental samples has been achieved. The method was based on the standard NOAA protocol. However, numerous improvements were made. The most impactful modifications made to the method allow the ability to achieve a lower size detection limit, 20 μ x 20 μ versus 300 μ, and to achieve a greater density limit, 1.8 g/L versus 1.2 g/L. In addition, novel microplastics data reporting techniques (2-D size and mass estimates) were developed in this project and will provide the research community much more valuable information than what has been previously reported.

Successful demonstration of the method was achieved by processing and analysis of microplastics in surface waters and in fish larvae. The method will be used as a starting point for further development of microplastic analysis methods for other media and will be used as a tool for microplastic monitoring programs.

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